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A Measure of Plant Response to Stress

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SYMPOSIUM PAPERS AND AUTHORS

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STRESS ETHYLENE PRODUCTION – A MEASURE OF PLANT RESPONSE TO STRESS. David T. Tingey.

J.F. Bartholic

D.W. Buchanan

A.D. Hanson

T.R. Sinclair

D.T. Tingey

INTRODUCTION TO THE SYMPOSIUM

D.W. Buchanan
Fruit Crops Department, University of Florida, Gainesville, FL 32611

This symposium sponsored by the American Society for Horticulturural Sciences and the American Society for Plant Physiologists is an effort to integrate plant microclimate and stress physiology research. The symposium was jointly organized by the Climatology and Meteorology and Developmental and Stress Physiology working groups. One objective is to discuss the proper instrumentation, its use, data acquisition storage and retrieval techniques for monitoring the plant microclimates and responses to changes in the environment, such as stress.

Complementary to this is a discussion of plant organ chambers used in field research, concepts of how plants respond to stress, and common indicators of stress.

ENVIRONMENTAL DATA ACQUISITION–A HISTORICAL PERSPECTIVE[1]

Jon F. Bartholic
Agricultural Experiment Station, Michigan State University, East Lansing, MI 48824

Much research in horticulture and plant physiology starts in controlled laboratory conditions. Plant parts are examined to obtain an in-depth understanding of processes. Later, plant experimental systems are moved to greenhouses or growth chambers, where the plant can be observed under more natural conditions. But again, the general environmental conditions of radiation, temperature, and moisture can be controlled. For findings to be of use to society, our research must ultimately move to the field, where control over the environment is lost.

Picture a peach orchard. The radiation intensity varies diurnally and with cloudiness. Wind velocity can vary continually from calm to very gusty. Temperatures of air, petals, twigs, and soil will all be different and vary with time. The humidity and moisture are also dynamic. At night, dew occurs first on petals and later on the twigs and trunk. Roots will be at a lower moisture tension than aerial parts of the plant. Varying amounts of carbon dioxide, ozone, sulfur dioxide, other pollutants, and pests will be present during an experiment.

Nearly all physical variables and many of the physiological parameter can be monitored. Monitoring these parameters provides a better understanding of what causes a particular plant response. For example, if we are modifying the micro-environment with overhead irrigation for freeze protection, exact temperatures of leaves, twigs, and fruit in the check and treated plot must be known. If rate and frequency of water application is variable, temperatures of leaves receiving different rates and frequency of water distribution must be known. The number of modifications that might need to be documented is infinite.

The underlying purpose of research endeavors was expressed by Lord Calvin: *"When you can measure what you are speaking about and express it in numbers, you know something about it, but when you can not measure, when you can not express it in numbers, your knowledge is of a meager, unsatisfactory counting."* As this quote expresses, it is important to document environmental conditions affecting our experiments. It is not satisfactory to know general conditions of cold/warm or dry/wet; rather, we need to quantify these values. It is now possible to quantify nearly all parameters in a data system that can be computerized for subsequently easy data reduction.

People and equipment

Documenting environmental conditions requires a certain "critical mass" consisting of people, instrumentation, and sensors to acquire the needed measurements. The most important factor is people–both technicians to help record measurements and colleagues to discuss the data. Technicians with electronic or computer backgrounds and a dedication to plant science research are essential. Considerable instrumentation and many sensors must always be used and maintained.

A certain minimum critical mass of sensors and computerized data acquisition capability is needed. Fortunately, computerized systems are gradually becoming less expensive and more reliable. The correct blend of critical mass keeps the scientist from getting bogged down with unmanageable data while handling the required measurements.

Good planning is essential before making measurements. The frequency and accuracy of measurements needed to answer particular questions must be clearly stated. The sensors for measuring those parameters can then be determined. The entire data system, from acquisition and recording to final data processing should be considered.

Sensors

There are many sensors that are available for monitoring the environment. These have evolved over many decades. The Gier and Dunkle radiometers first appeared in the early 1950s (2). Subsequently, these radiometers have been improved in reliability, accuracy, and stability (1). Similar improvements have been made for sensors used to measure nearly every environmental parameter. Environmental contamination measurements can now be made with sensors that have fairly high reliability. Nearly every campus or large research group has personnel capable of guiding other scientists in sensor selection.

Basically, all sensors are designed to measure physical quantities. The desired physical quantity is sensed by monitoring changes in properties of the sensor. For example, as temperature increases, resistance of most metals increases. This resistance change can then be related to temperature. With thermocouples and many radiometers, a voltage output is proportioned to the quantity being monitored. Some anemometers have a voltage output while others provide pulses which can be integrated over time to give the total wind movement (wild run) for the interval. A simple division by the time interval gives the average wind speed.

A schematic diagram for a linear variable differential transducer circuit, to measure tree shrinkage due to water stress, is shown in Fig. 1. This measurement indicated the growth of a tree from day to day (3,5). One basically has *the sensor* attached to a tree, *circuitry* powered by AC voltage that excites the transducer and demodulates the subsequent signal with a D/C *outputvoltage* to a *recorder*. With nearly all measurements one has the sensor, electronics, and a signal output that can be monitored (recorded) over time.

Recorders

A simple strip chart recorder is designed to trace a signal over time. Such devices, in use for decades, are ideal if only a few parameters are being measured and recorded. The thermograph, or hygrothermograph, is perhaps the simplest and most common recording system. A bimetallic temperature sensor moves an inked pen which traces on a rotating drum chart. The significance of such simple and dependable devices should not be overlooked.

Recorders can play an important role in data acquisition. But in experiments where numerous parameters are measured, several recorders are required. The recorder data must all be reduced by hand, which can take weeks or months if numerous detectors and recorders acquired data over a summer period. A management problem also exists in reducing chart data which often have variations in time base, missing data due to voltage outages, printer ink running

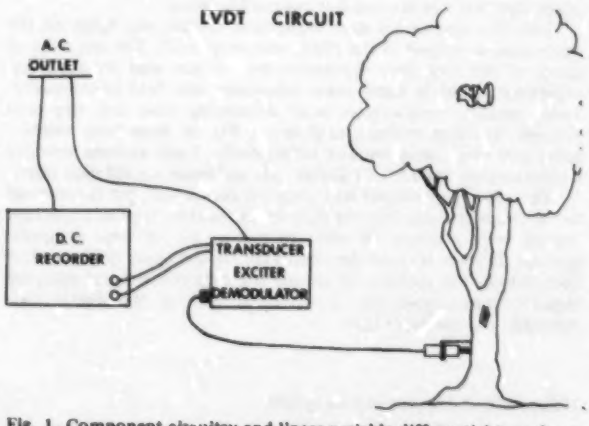

Fig. 1. Component circuitry and linear variable differential transducer (LVDT) for continual measurement of tree shrinkage due to water stress and growth.

[1]Publication No. 9287 of the Michigan Agricultural Experiment Station.

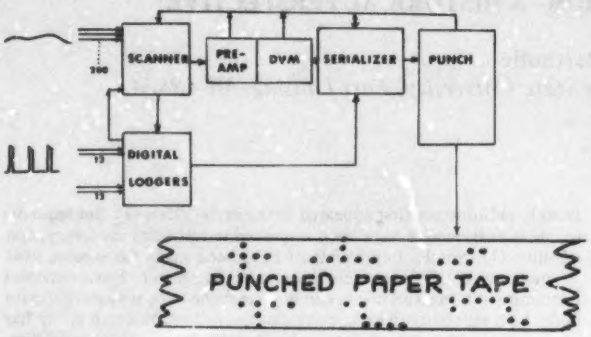

Fig. 2. A block diagram of the components and flow of data in a first generation data acquisiton system (from sensor volgate or pulse to digital punched paper tape. DVM = Digital Volt Meter.

out, and numerous other problems. The time period between running the field experiment and analyzing the results can be 6 months to a year, making it difficult to know if the data acquired are of high quality.

In the early 1960s, other electromechanical devices were used. The pulses from anemometers placed at various heights provided a wind profile. These pulses could be transmitted to electromechanical counters. The electromechanical counter could be manually read or more elaborate systems could print out data periodically. One ingenuous device was to have the counters in a samll box with a Polaroid camera periodically snapping pictures of the values on the electromechanical counters. This data acquisition method was specifically geared to unique sets of measurements, but many such examples could be cited.

Data acquisition system

Numerous individual units (recorders, counters, etc.) were too cumbersome to be an effective approach for documenting the environment. The need for an integrated approach led to a first generation data acquisition system. A flow diagram for this system is shown schematically in Fig. 2. The analog signals, or pulses, from anemometers came from the left to the data acquisition system. The scanner frequently took up to 200 analog signals from thermocouples, strain gauges, or other devices. The number of pulse counts in the system could be expanded, depending on the anemometer number. The scanner routed the analog signal into a pre-amplifier. Thus, one pre-amplifier was used to multiply each signal level. A digital volt meter (DVM) converts analog signals into a digital value that can be read on the digital meter.

The digital data had to be serialized and sent one digit at a time to a punch to be recorded on a paper tape.

This system allowed multiple signals, either analog or pulse, to be scanned by the same system and recorded immediately onto a punched paper tape that was in computer compatible format.

This first generation data acquisition system was based on the electronics developed in the 1950s and early 1960s. The reliability of many of the first data acquisition systems not only for biological experiments, but in many other laboratory and field environments. Thus, many manufacturers kept developing new and improved systems. The first systems, as shown in Fig. 3a, were fairly cumbersome and some never worked satisfactorily. Later systems provided a considerable amount of valuable data and some are still used today.

The paper tape output was computer compatible, but it could not be read rapidly. And frequently, only the crudest type of paper tape readers were available (if they existed at all) on large computer systems. It soon became clear that even though many problems had been solved, the problem of getting the data reduced and analyzed required time-consuming steps beyond getting the digital data punched onto the paper tape.

Computerized data acquisition system

Laboratories throughout the country were demanding that computers be part of the data acquisition system. This became possible with the advent of computers designed in the mid-1960s. Data acquisition could not only be controlled by these computers, it could also be fed back into the system for reduction and analysis.

The computers available in the mid-1960s were bulky. Solid state chips were not available. Ferrous core memory was used and systems were too large to allow mobility. An example of an early system designed for data acquisition is the IBM 1800. It was used for data acquisition and control in oil fields and many large industrial plant operations. These systems demonstrated the usefulness of comput-

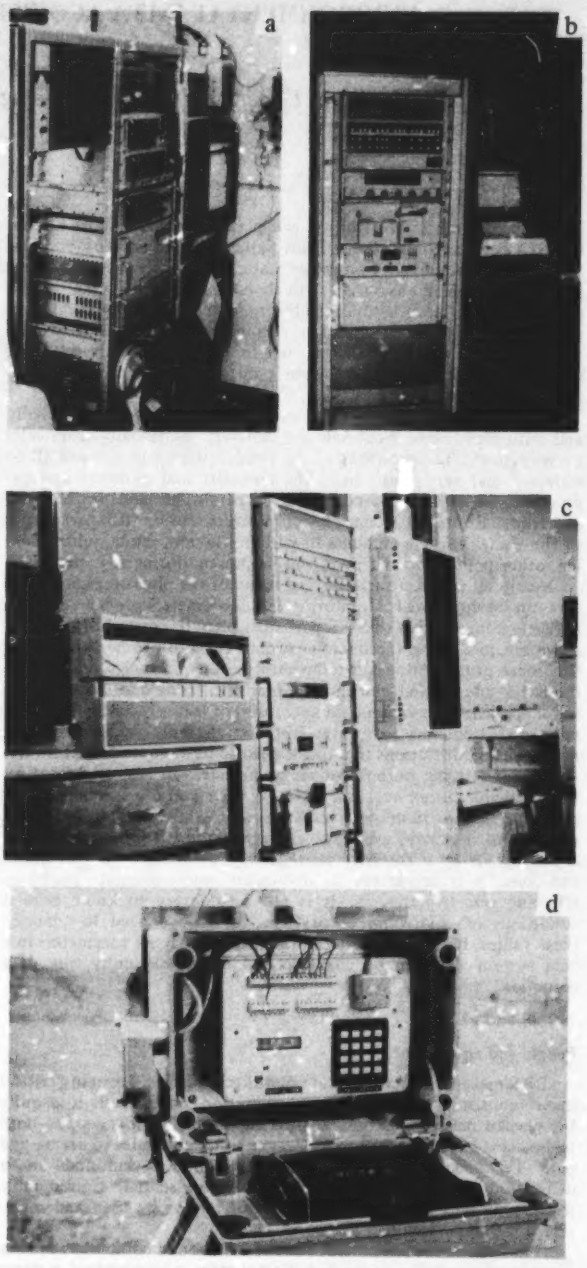

Fig. 3. a. First generation data acquisition system. b. Computerized second generation data acquisition system. c. Third generation computerized data acquisiton system with disk and digital magnetic tape. d. Microprocessor controlled data acquisiton system.

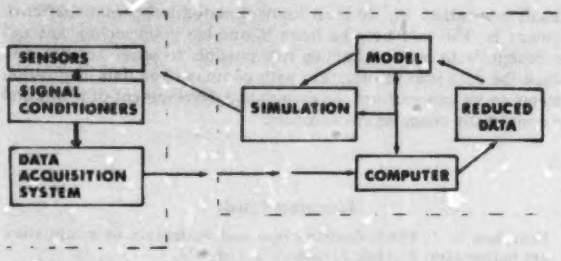

Fig. 4. Flow diagram for data acquisition, reduction, and analysis.

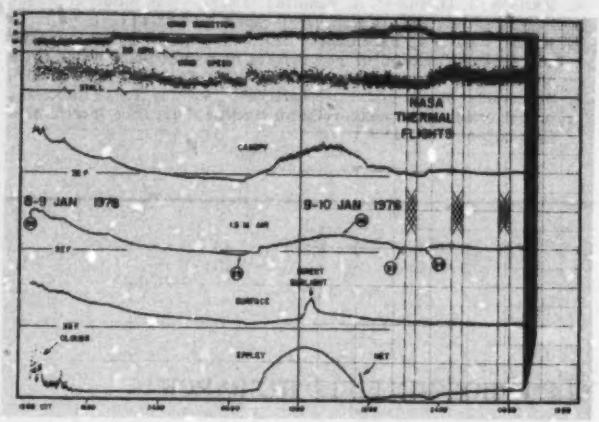

Fig. 5. Photograph of plotted output from third generation computerized data acquisition system. Surface temperature shows "unusual" discontinuity near midday.

erized data acquisition, but, for the horticulturalist, they were too cumbersome and expensive for consideration in real time applications.

The continual reduction in computer size and cost soon made it possible to have mini-computers that were compatible with constraints of the data acquisition environment. These systems were small enough in the late 1960s to be rack-mounted with the previous parts of the data acquisition system; i.e. digital volt meter, scanning terminal punch (Fig. 3b). The punch in this case was seldom used. Rather, the data were analyzed and reduced by the computer into engineering units, fluxes or other information needed, and it was reported directly in written format on a terminal. This second generation system has proven to be a very effective unit for horticultural and physiological experiments.

One shortcoming of the system was inflexibility. Once this system was programmed it would work fine for that application, but the computer was not accessible for reprogramming while it was acquiring data from the experiment. Thus, shutdown time was required for program modification and between experiments.

Third generation: computerized data acquisitions system

Because of the modular nature of computerized data acquisition systems, it was possible to add peripherals and improved software operating systems to upgrade to the third generation system. The hardware peripheral additions included a disk and possibly digital magnetic tape capability (Fig. 3c). The disk made random access memory available to the computer. New software operating systems allowed both foreground and background operations in the computer. This system allowed routing data acquisition in the foreground while, in the background, programmers could develop new programs or modify existing programs for future use in the foreground. This allowed for a dynamic computerized data acquisition system. Also, data acquired in the foreground could be easily transferred to the background for further analysis.

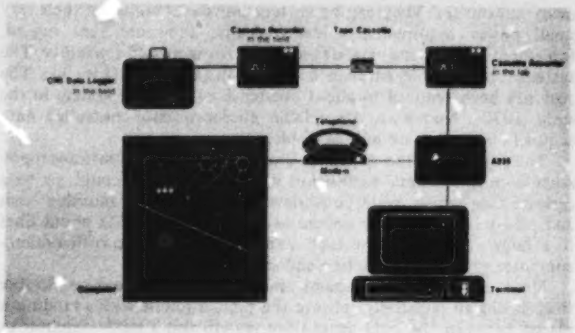

Fig. 6. Schematic diagram of operational flow of data rate using a microprocessor for the field data acquisition.

A schematic diagram of this complete, perhaps ultimate, third generation computerized data acquisition system is shown in Fig. 4 (4). On the left are the basic components shown in earlier systems for sensor signal conditioning and data acquisition. However, rather than going to paper tape, data can be reduced and printed out so the scientist can see exactly what is happening in real time. Also, small models could be used in the background and real time data could be used in these models so energy fluxes, predictions of growth, or other responses could be predicted and then checked against actual measurements. By closing this loop of data acquisition, and by reduction and use of the data in models, it is possible to go back in nearly real time and revise sensor configuration or locations so that the experiment could be carried on in an improved manner if that was deemed to be appropriate by the scientist or his technicians after examining the computer-digested information.

Options such as a plotter could eaily be added to this system for better visual interpretation of response versus time. One example of this is to plot key parameters over several days, since the test location might not be visited each day. When one came in several days later, the records could be easily examined in a few minutes to see if all sensors and information were working properly. Also, since there is so much information that could be acquired, the plots allow for more rapid examination of a large volume of information.

An example covering 2 days is shown in Fig. 5. Examining the surface temperature with time, the termperature between the rows of citrus trees warms up slightly with time, but at midday a peak occurs (6). Our initial reaction was that these were erroneous data. However, since it was plotted in real time, we could look at what was happening in the field simultaneously with the measurements. Over this particular short interval the solar radiation was not being shaded by the N-S oriented rows of citrus, but was directly reaching the soil surface between the rows. Thus, the reading was correct and we were just getting a large radiation load for a short time on the soil between the trees. This is one example of why it is helpful to have the data plotted in a form that allows observation and evaluation in real time.

Because of the flexibility of the system, many programs could be developed in the background for doing other tasks. For example, the calibration of sensors could be checked and the system could be programmed to open and close relays for controlling equipment or the temperature in chambers.

Again, with new technologies in this third generation system, data could be fed to other computers through phone modems, or terminals could be used to call up the data acquisition system in the field, allowing a scientist in his office to be sure that data were being acquired correctly.

We have found this capability to be very valuable in freeze studies. Normally, we would have to have 1 or 2 technicians on the site observing output from a data acquisition system. However, with the phone couplers it is possible to take a portable terminal home and periodically check temperatures in a grove via phone. This saves considerable time at the site, reduces travel, and increases project productivity.

Fourth generation: microcomputer–data acquisition systems

Conceptually, the microcomputer is nearly as powerful as the mini-computerized data acquisition systems. The microcomputers have

many advantages. They can be battery powered because of their very small power requirements. Electronically, they are very rugged. Integrated circuits and new design make for increased reliability. The systems are portable and can be placed in field plots (Fig. 3d). The cost has been reduced to about one-tenth of a similar system in the early 1970s. Numerous dependable microprocessor-controlled data acquisition systems are now available.

The concept of the system is shown in Fig. 6. The suitcase represents a portable data acquisition system. The data could go to a cassette tape which could be retrieved on a weekly or monthly basis and read into a computer system in a laboratory and via phone lines to a large system. Or, the tape could be read into an office microcomputer system for reduction and analysis.

More commonly, 2 modums and a phone line are used. Rather than having to physically retrieve the tape, a phone with a modum is placed between the data acquisition system and a mini-computer or large computer system. The larger computer system can then hourly or daily call the microprocessor and query the data in the system.

Conclusion

We need to quantitize if we are to truly understand and talk in a sensible way about research findings. Since most of what we study outdoors is strongly influenced by the environment that plants and animals are bathed in, we must know quantitatively what that environment is. This can now be done if one has good technicians and equipment. With proper planning it is possible to sense, acquire, and reduce the data into information with computerized data acquisition systems so we can measure the growth and development of plants and the continually changing environment.

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PLANT ORGAN CHAMBERS IN PLANT PHYSIOLOGY FIELD RESEARCH

T. R. Sinclair

University of Florida, Gainesville, FL 32611

With the advent of reliable and accurate gas analyzers, plant physiological performance can be observed by changes in the exchange rates of specific gases between plant tissue and the atmosphere of a plant organ chamber. This paper considers the use of plant organ chambers, in conjunction with environmental measurement and control techniques discussed in previous papers in this series, to do field physiological research. The use of these chambers has considerable appeal because, at least superficially, it is relatively simple to enclose a plant organ in a chamber and monitor its response. However, the appeal of these chambers is offset by the inherent limitations existing in their use in field physiological research. Three limitations that are discussed here are 1) artificiality of the system, 2) ambiguity in data interpretation, and 3) commitment required to obtain "adequate" information.

Nevertheless, these limitations do not necessarily mean that no useful information can be obtained from plant organ chambers. In the latter part of this paper 2 examples of the use of chambers in physiological research are briefly reviewed. A simple, rugged leaf chamber design is discussed first. This chamber was developed for use under field conditions to monitor on a long-term, continuous basis the changes in the exchange rates of CO_2 and water vapor by leaves. Second, a system for making *in situ* measurements of N_2 fixation rates is presented. This system employs the acetylene reduction approach for following N_2 fixation and was also designed to work under field conditions for long periods.

Inherent limitations of chambers

Artificiality. By definition, if a plant organ is enclosed in a chamber, an environment differing from the one in which it normally grows has been imposed. The chamber restricts gas flow, so that a new and different pattern of air flow develops around the organ. In addition to an altered air flow, the concentration of gases in the chamber atmosphere will adjust to new levels. Substantial changes in atmospheric humidity levels, for example, are probably the most frequently encountered when enclosing plant material which evaporates water vapor. The change in gas concentrations are, of course, frequently required if measurements of gas fluxes are to be made. In the chamber other environmental changes occur, such as the quantity and quality of the irradiance, air and organ temperatures, and alteration of organ exposure to the usual physical and pathological stresses. Unquestionably, even with the best of designs, the chamber environment is never the same as the natural environment of the organ.

Not only is the chamber environment uniquely different, the plants studied within chambers are invariably given special treatment to facilitate the research. The most dramatic examples of this are the agronomic and horticultural crops naturally grown in fields but which in physiological studies frequently are grown in greenhouses and growth cabinets. Several recent studies have shown marked differences between the growth and physiology of plants grown under controlled conditions and those grown in the field (e.g., 1, 5, 8). Therefore, considerable care must be used in extrapolating the results obtained with plants grown under controlled conditions. Even when plants are studied in the field, artificialities are also introduced. Special row spacing to facilitate the research and abnormally greater soil compaction around the plants are 2 possibilities.

Finally, an artificiality that is the most difficult to resolve in data collected from plant organs in chambers is the induction of plant behavior that is a response to being placed in a chamber, This problem is a fundamental dilemma of chamber research—what assurances are there that the experience in the chamber does not upset "normal" physiological function? As an example, a possible artificiality in leaf photosynthetic research, may result from the desire to minimize the interference of a leaf boundary layer resistance. Wind speeds around the leaf can be maintained at very high levels—internal fans are sometimes employed—and the experimenter waits for steady-state conditions to develop. Under these conditions it is easy to visualize unusually high transpiration rates which dehydrate the leaves. Stomatal adjustments under these conditions can reduce photosynthetic rates and, thereby, produce rates that are lower than those normally occurring under natural conditions. Other feedback or feedforward scenarios can be readily suspected for many physiological events in plants subjected to chambers. Caution seems essential before extrapolating any data beyond the circumstances under which it was collected.

Ambiguities in interpretation. Once accurate data are collected from a plant organ in a chamber, there may be considerable difficulty in interpretation of the data. These data will reflect the integrated ability of the intact organ to exchange gas, which may be a consequence of a series of complicated processes. Interpretation of results in terms of the performance of a single process or enzyme event may be extremely difficult. For example, in measurements of intact-nodule N_2 fixation by the reduction of acetylene to ethylene both physical diffusion and biochemical activity are involved. Estimating from these data the activity of nitrogenase during N_2 fixation may be difficult.

Also, analysis of data by generalizing to the performance of a community of organs is difficult. Assurances must be developed that the behavior of a few individual organs are representative of nearly all organs. For example, the photosynthetic response of an individual leaf may be measured, but what is the response of an entire leaf canopy? In the leaf canopy, leaves exist that developed under differing environments and have varying ages, positions, and microenvironments. It would seem that fully characterizing a community of organs may require studies of greater and greater detail not justified by an original objective of knowing the broad performance of the community. Certainly, ambiguities in the generalization from individuals would need to be verified, possibly by even larger and more complex chambers.

A final problem in the interpretation of chamber data is the accumulation of sufficient data by observing large numbers of individuals, and adequate sampling in terms of both chronological and ontogenetic age. The criteria for sufficiency will, of course, depend on the objectives of the research. Nevertheless, it has become increasingly clear in field physiological studies that more attention must be given to possible changes that may occur with age. Also, associations almost certainly exist between the age of the individual organ and the life stage of the entire plant. A further complication that has received minimal attention has been the influence of environmental sequencing and variability on physiological performance. How long do the results of meterological events persist in plants, and are these changed by differing environmental scenarios and plant growth stages? Rarely has it seemed that too many data have been collected for the interpretations and extrapolations generally attempted with the data.

Commitment required. To minimize many of the limitations discussed above, a substantial commitment in using plant organ chambers in physiological research must be made. As in any research, a clear definition of the research objectives is essential. The extent to which compromises are acceptable should be considered carefully. The requisite equipment and personnel must be assembled, and these are generally fairly specialized and expensive.

In developing chambers for physiological field research, considerable attention needs to be given both to monitoring and controlling the microenvironment of the chambers. With the advent of inexpensive minicomputers and microprocessors, systems can be designed to give real-time control of the chambers such that the natural environment is closely mimicked, or fairly complex changes independent of the natural environment can be programmed. The use of microprocessors has also allowed nearly continuous, long-term observations of a number of chambers. While such equipment advances greatly increase the scope of the data collected, it, at the same time, requires that the experimenter must make a substantial long-term commitment of his time to develop the systems and to monitor performance. Long-term observations mean both long-term commitment to a vigilance that good data are being collected, and to analyzing and resolving the large amounts of data that are accumulated. Our knowledge of the complexity of the physiological systems being studied, and the power of modern equipment have outstripped the concept of collecting only a few samples to roughly estimate the performance of plant organs.

Examples of plant chambers

Leaf photosynthesis chambers. Considerable attention has been given to the various approaches and difficulties in constructing chambers to make measurements of leaf photosynthesis. In fact, Sestak, et al. (9) edited an entire book dealing with methods involved in measuring photosynthesis. Several systems developed more recently have sought to greatly simplify the design of chambers, yet minimize the deviation in the chamber environment from the natural one (6, 10). In this section, some of the considerations in the development of the chamber used by Sinclair et al. (10) are reviewed.

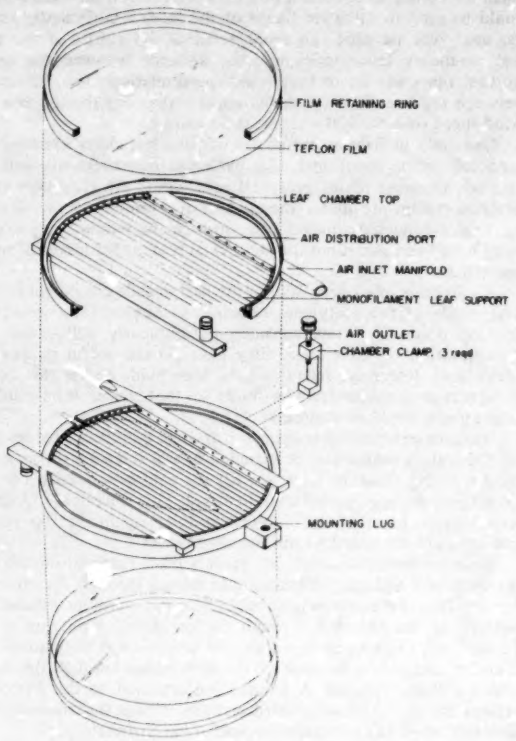

Fig. 1. Projection drawing of top and bottom parts of leaf chamber used to make continuous, long-term measurements of leaf gas exchange (from 10).

A projected drawing of this leaf chamber is shown in Fig. 1. The design is simple, with the chamber frame being constructed from 2 slices from free cutting brass tubing, either 15 or 18 cm in diameter. Leaves are held between the 2 frames by monofilament lines threaded through holes in each frame. Air distribution manifolds were soldered into each frame so that air would pass over both surfaces of the leaf. The main surface of the chamber is FEP Teflon film, 25 μm thick, which is simply held in position by retaining rings that fit in the chamber frame. A 1.6-mm thick neoprene gasket is attached to the frame, forming the bottom portion of the chamber. When the 2 frames are clamped together, an air-tight seal is formed, resulting in a simple, rugged leaf chamber.

The main environmental design criteria for this chamber were a) natural irradiance, b) minimum heat build-up, and c) small gas concentration change. The FEP Teflon film proved particularly suited for giving near-natural irradiance. This film is more than 90% transmissive in the 0.3 to 7.3 μm range and at least 50% or greater transmission in the 9 to 15 μm band. The added benefit of having FEP Teflon as the major surface of the chamber is that it is inert to a wide range of materials.

To help prevent temperature increases in the chamber, it was constructed with minimal thermal mass and all metal parts were chrome plated to give high reflectivity. Heat removal in the chamber was accomplished by a high volumetric flow. Under most circumstances the air flow was held constant at 250 cm^3/sec. If the sensible heat load was as high as 18 mW/cm^2 for a 50 cm^2 leaf, the total temperature rise of the air exiting the chamber was still only 3°C. Immediately before entering the leaf chamber, the air flowed through a heat exchanger that generally kept the air entering the chamber at or slightly below ambient temperature. The temperature of the leaf was monitored with a 4-junction thermopile sprung against the underside of the leaf.

The high volumetric flow also caused the change in concentration of carbon dioxide and water vapor in the chamber to be small. The small difference in concentration between the entrance and exit ports could be used to calculate fluxes of the gases if sufficiently accurate gas analyzers are used. To avoid potential difficulties of too small a leaf boundary layer resistance, the distance between the top and bottom films was set to keep wind speed relatively low. The distance between the two films for the closed chamber was about 5 mm so the wind speed over the leaf was about 30 cm/sec.

Generally in field operation, 39 of these leaf chambers were simultaneously being monitored. Gas exchange measurements were made on each chamber about every 10 min. Since the chambers and gas analysis system are under the control of a minicomputer, data were essentially collected continuously through the season. CO_2 exchange rates have been measured during several seasons for leaves of soybean and citrus.

N_2 fixation chambers. Within the past decade considerable use has been made of the acetylene reduction technique for estimating N_2 fixation potential. While considerable ambiguity still exists in the interpretation of these data, they have proved useful in qualitative evaluations. Recently, attempts have been made to use the acetylene reduction in a nondestructive mode so that longer term studies on intact plants could be undertaken (3,4, 7).

We have extended this approach to field conditions by developing root chambers which can be installed in soil and used for season-long studies of N_2 fixation rates; see (2) for complete details. Fig. 2 is a drawing of the open-ended chamber used. This chamber is 15 cm deep and, because most of the soybean nodules are in the top 10 cm of soil, they are contained within the chamber.

When observations are being made a lid is clamped on the top of the chambers and gas is continuously passed through the chamber at 1.7 cm^3/sec. Air containing about 0.2% aceytlene is introduced at the bottom of the chamber through the gas distribution ring at about 17 cm^3/sec. Consequently, an air seal is formed at the bottom of the chamber and only a fraction of the air is passed through the chamber under a slight vacuum. A sample line attached to the chamber lid returns the gas to an automated analysis system that measures acetylene and ethylene concentrations with a gas chromatograph.

The main design criteria for this chamber included a) minimum interference with normal plant growth, b) minimum disruption of normal N_2 metabolism and c) minimum concentration of ethylene. These root chambers are installed in the soil when the rest of the field is seeded. Seeds germinate readily in the chambers and the roots quickly grow out the bottom of the chamber to explore the soil for water and minerals in the normal fashion. The plants grown in the root chambers were found to have growth patterns essentially identical to the rest of the field plants.

Generally, experiments using the acetylene reduction technique have used saturating concentrations of acetylene. While, for short-term observations, this may be satisfactory, during long-term studies the complete elimination of N_2 fixation will almost certainly disrupt normal physiological activity. The loss of fixed N_2 may decrease plant activity, or, on the other hand, the reduction of any possible negative feedbacks by the products of N_2 fixation may increase activity. Therefore, the disruption of N_2 fixation is minimized by keeping acetylene concentration much below saturating levels, i.e. at about 0.2%. Extrapolation of the data to the saturating acetylene concentration was accomplished by using the Michaelis-Menten approximation. This approach allowed plants to be successfully monitored throughout the season with no apparent effects on growth and development.

Ethylene is a potent plant hormone which can alter plant growth and physiology. However, only small amounts of ethylene are produced because of the low concentrations of acetylene that are used. To maintain low concentrations of ethylene, a continuous flow chamber was developed. With a flow rate of 1.7 cm^3/sec, the volume in the chamber is completely flushed out about every 5 min and the concentration of ethylene in the chamber is rarely greater than 10 µl/liter. In soybeans, no effects on plant growth and physiological function that suggest ethylene activity have been observed, even when plants were studied all season long. The added benefit of the continuous flow system is that it allows gas samples to be readily collected and analyzed at frequent intervals.

This system of root chambers has also been placed under the control of a minicomputer. Every 30 min gas exchange data are recorded for each of the 40 root chambers that can be studied at one time. This system allows continuous observations of plants so that diurnal, weekly, and seasonal changes in N_2 fixation rates can be observed.

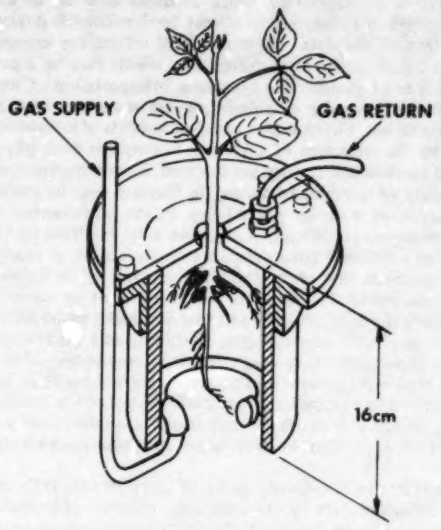

Fig. 2. Projection drawing of root chamber used to make continuous, long-term measurements of N_2 fixation rates by acetylene reduction (from 2).

Summary

These 2 examples of plant organ chambers used for measuring gas exchange demonstrate that with due concern for the chamber environment and for the normal growth of the plants, useful data on physiological performance under field conditions can be collected. Recent advances in electronics, particularly the development of minicomputers and microprocessors, have greatly expanded the potential for monitoring and controlling plant organ chambers in field physiology research. These tools allow the scope of the research to be considerably broadened because many chambers can be observed essentially simultaneously and continuously on a long-term basis. The inherent limitations of artificialities and ambiguities in the data can be minimized by good control of the chamber environment and a multiplicity of chambers. While these technological advances allow intensive field physiological research, they also require a substantial commitment from the experimenter. During the data collection, a continuing, long-term effort is required to assure high quality data. Having completed the data collection, the experimenter is confronted with a very large volume of data that must be analyzed and interpreted. Yet, the rewards of these commitments appear to be an ever-increasing understanding of the physiological processes existing in plants grown under field conditions.

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INTERPRETING THE METABOLIC RESPONSES OF PLANTS TO WATER STRESS[1]

Andrew D. Hanson[2]
MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Climatic factors–particularly the ranges and mean values of water availability and temperature–were major selective forces in the evolution of crop plants, and it is adaptation to these factors which sets the broad distribution limits for today's crops. The *extremes of the ranges* of water availability and temperature (the *stresses* of drought, flooding, cold and heat) likely to be encountered at any location are particularly important in determining the crop species and cultivars which can be profitably grown there, i.e. the *adapted genotypes* for that environment. The phenomenon of plant adaptation to environment can, in principle, be understood in physiological, biochemical and genetic terms. Current research on crop adaptation to stress-prone environments, research which seeks such an understanding, may influence the future practices in both plant breeding and crop management.

Drawing for examples upon adaptation of cereals to drought-prone environments, this paper covers 4 fairly general issues in research on crop adaptation to stresses:

1) The unexploited variation in adaptation which may exist in the gene pools of crop species and their relatives.

2) Metabolic pathways as components of adaptation, and as symptoms for detection of stress injury.

3) Whether 2 metabolic responses to stress–proline and betaine accumulation–may be adaptive events or injury symptoms.

4) The potential for applying metabolic tests for stress–adaptation in plant breeding.

The following recent publications treat crop adaptation to various types of environmental stresses (1,2,5,7,9,15,16,18,19,21,22,31).

Genetic variation in adaptation to stress

It is probable that among cereal crops and their relatives, those genotypes originating from locations prone to drought stresses carry specific adaptive traits which could be exploited in plant breeding (e.g. 17,34). These traits can be either constitutive (always expressed) or inducible (expressed only in the presence of stress).

The sorghum breeding program at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is seeking to detect and utilize such adaptive traits; a striking illustration of their potential value is given by the ICRISAT data replotted in Fig. 1. A number of diverse sorghum genotypes, with grain yield potentials in well-irrigated conditions covering a 3- to 4-fold range (X-axis) were subjected to a mild (Fig. 1A) or a severe (Fig. 1B) pre-anthesis drought. The mild drought stress reduced the yields of all genotypes by about 20%, so that grain yield after stress (Y-axis) remained highly correlated with yield potential in the absence of stress. Two corollaries of this high correlation are: 1) that *environmental* measurements of the mild stress should enable fair prediction of yield for any genotype whose yield potential in well-irrigated conditions is known; 2) that in production environments usually subject only to mild stress it is *not* worthwhile to breed for drought-resistance *per se*, because the yield performance of a genotype in well-watered environments is a good predictor of its performance under mild drought.

Severe stress provoked very different responses among genotypes with similar yield potential (arrows), so that the correlation between grain yield after stress and yield potential became much weaker. Two consequences of this for severe stress situations are: 1) that it is not possible to make reliable predictions of genotype performance from environmental measurements alone; 2) it becomes desirable to breed specifically for drought-resistance. Evidently, severe stress provokes expression of a wealth of genetic variability, and various adaptive traits become important determinants of both growth and yield, because changes in both biological yield and harvest index contributed to the yield effects shown in Fig. 1B (14). Similar cases have been documented for other cereals, e.g. barley (6) and wheat (17).

The existence of adaptive traits or characters whose presence or absence becomes apparent only during stress raises 2 questions: 1) Can these characters be identified and exploited in plant breeding? 2) Can the expression of these characters during stress be used to monitor plant performance and to improve the precision of yield predictions? The next section examines how studies of stress metabolism may help to provide answers.

Metabolic components of adaptation, and metabolic symptoms of stress injury

Adaptation to drought-prone environments can be viewed as the sum of traits expressed at several levels (Table 1). Much genotypic variation in response to drought-stress of the sort seen in Fig. 1B is often attributable to phenological characters, and sometimes to morphological traits (e.g. 1). Both phenological and morphological traits are now used in crop breeding for adaptation to drought as supplements to the criteria of yield and yield stability. Adaptive physiological and metabolic traits certainly exist (1) but have so far found very little and no application in breeding, respectively, because they are as yet insufficiently described and understood.

Indicators of plant performance during stress–"stress symptoms"–can also be expressed at various levels (Table 2). Here again, phenological and morphological markers are now used in both breeding and crop management. Physiological stress symptoms are also finding quite wide application. Metabolic stress symptoms have no present uses, again because they are poorly characterized–not because they do not exist.

In order to exploit metabolic pathways either as components of adaptation or as stress symptoms, first steps are to describe the behavior of crop species and cultivars with respect to metabolic responses to stress, and to interpret the physiological significance of these responses. Metabolic responses might be classed in the following way:

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[2]I wish to thank Drs. E. H. Everson, J.E. Grafius, J.F. Fobes, and N.E. Good for many discussions and for their valuable reviews of this paper.

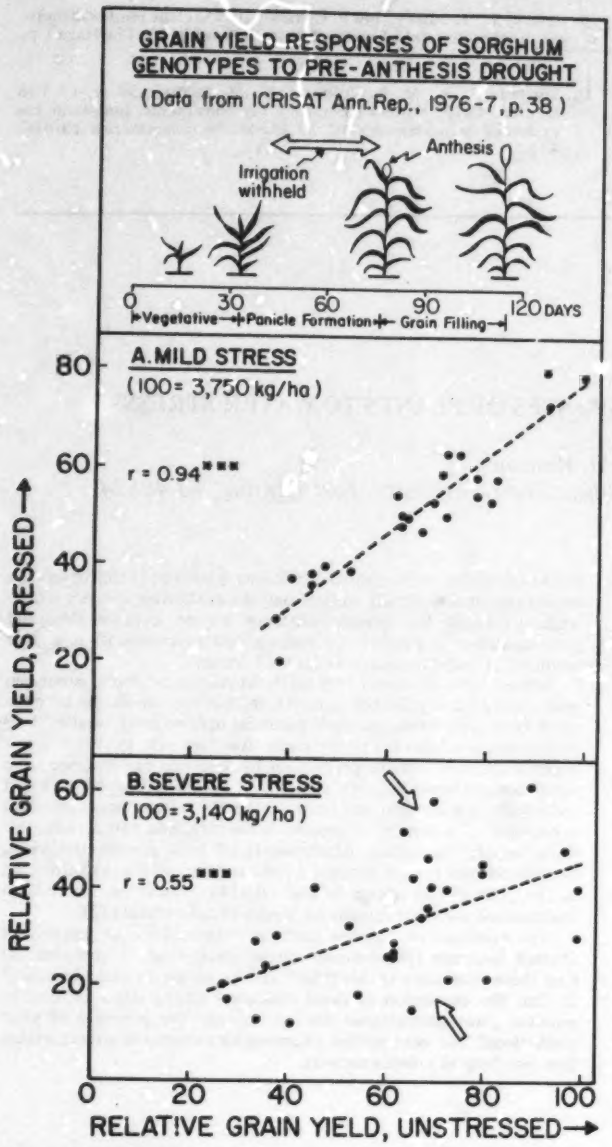


Fig. 1. The relationships of sorghum grain yields under stress with yields in absence of stress. Irrigation was withheld during the panicle formation stage in experiments in the hot, dry summer (Fig. 1B) and in the cooler post-rainy season (Fig. 1A).

a) *Adaptive:* i.e. responses which favor survival, growth and yield under stress.

b) *Deleterious:* i.e. responses which compound the stress, and reduce survival etc.

c) *Incidental:* i.e. responses which accompany stress, and are essentially neutral in their effect.

In principle, plant breeding programs could identify sources of desired adaptive responses, and then practice selection *for* such responses; alternatively, selection could be *against* deleterious or incidental responses. Crop management might seek to maximize the expression of adaptive responses, and to minimize expression of the other 2 types. The following section examines some recent work on 2 metabolic responses to stress from the standpoint of genetic variability for the occurrence of each response and from that of their physiological significance in stressed crops.

Table 1. Some heritable characters that may confer adaptation to drought-prone conditions.

Level of adaptive character	Examples among cereals
Life-cycle or phenological	Early maturity in winter and spring wheats of U.S. and Canada. Late maturity in upland rices.
Morphological	Rooting patterns in Canadian spring wheats. Awns in wheat. Surface waxes in sorghum.
Physiological	Diurnal and seasonal osmotic adjustment in sorghum, corn, wheats. Stomatal behavior patterns in rice, barley, wheats.
Metabolic	?

Table 2. Some plant parameters (= symptoms) that may enable detection and/or measurement of water stress during growth.

Level of parameter or symptom	Examples among cereals
Life-cycle or phelological	Tiller development Delay or advance in anthesis date
Morphological	Leaf rolling Leaf-tip firing Reduction in plant height and leaf size Wilting
Physiological	Stomatal resistance Water potential and/or turgor potential Leaf temperature
Metabolic	?

Proline and betaine accumulation

Free proline accumulates in the leaves of very many species subjected to water stress, to salination, and to low temperatures (e.g. 3,4, 20,23,27). Water stress and salt-stress also elicit betaine accumulation in leaves, but this response occurs in a narrower range of species than proline accumulation (e.g. 8,28,32). There has been some speculation about the possible adaptive significance of proline and betaine in mesophytes and halophytes (e.g. 24,33), and proline accumulation has been proposed both as a criterion in breeding cereals for drought-resistance (23) and as a symptom for measuring crop water stress (20).

Barley accumulates both proline and betaine in response to stress, and much metabolic work on these responses has been with barley seedling leaves. The pathways involved in proline and betaine accumulation in barley are summarized in Fig. 2. Fig. 3 shows typical changes in proline and betaine levels which occur in the leaves of intact barley plants subjected to progressively increasing water stress. Similar results are obtained whether the stress is imposed by withholding irrigation or by flooding roots with a solution of polyethylene glycol.

Proline accumulation. Among the Gramineae, there is probably no *gross interspecific* variation in the proline accumulation response; proline levels before stress are always low and no non-accumulating species are known. Thus, free proline levels in the range 1 to 5 μ mol/g dry weight are found in the absence of stress for many cereals and grasses, including rice, sorghum, corn, wild and cultivated wheats and barleys, and *Agropyron* spp. Upon imposition of stress, seedlings of all these genotypes start accumulating proline when leaf water potential (ψ_{leaf}) falls below about −10 bars. After a few days of wilting, 20- to 100-fold increases in proline content are common. There is much *fine inter- and intraspecific* variation in the extent of these increases (e.g. 10,24).

Four main changes cause free proline to accumulate under stress (Fig. 2). First, there is a stimulation of proline synthesis from glumatic acid, perhaps due to loss of feedback inhibition of the synthesis of the intermediate Δ^1-pyrroline-5-carboxylate (P-5-C). Second, proline

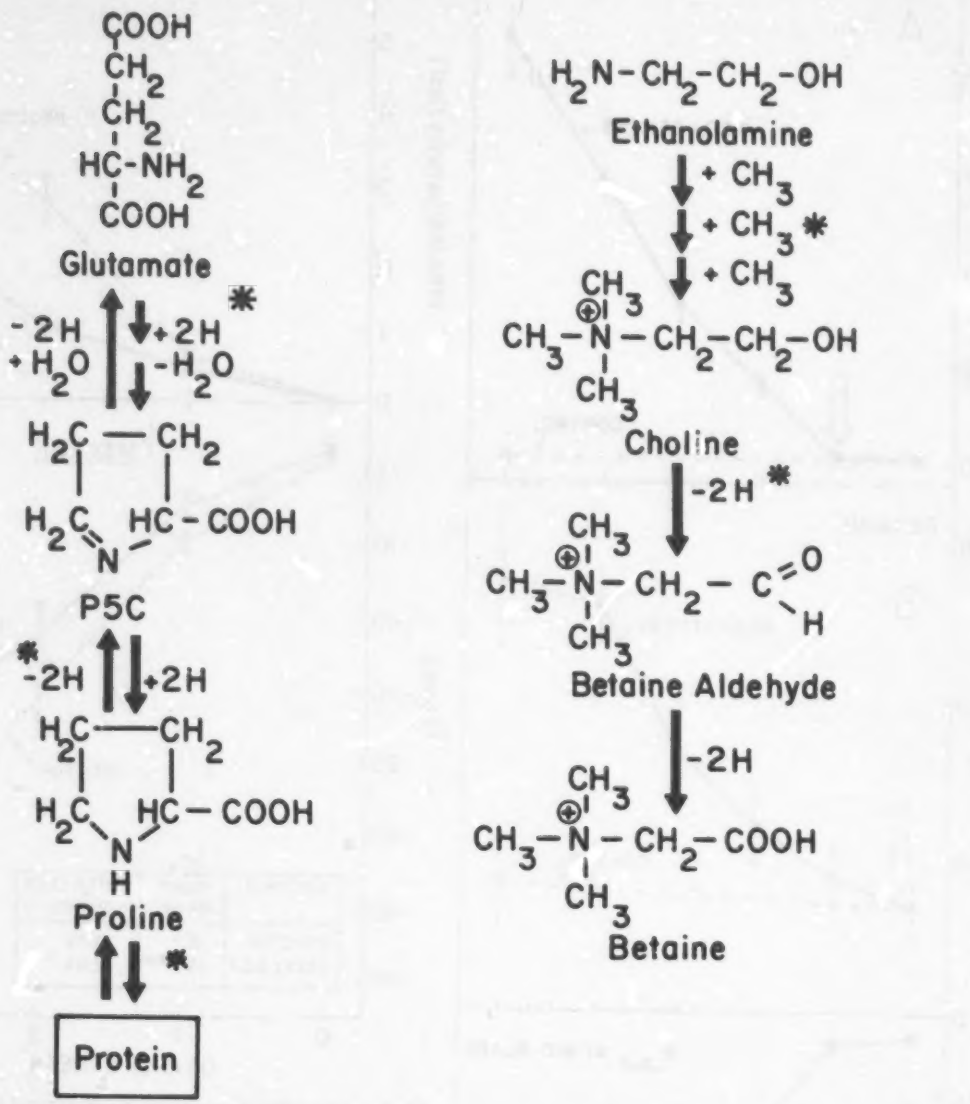


Fig. 2. Metabolic pathways involved in proline and betaine accumulation in water-stressed barley leaves. Asterisks designate metabolic steps which are probably involved in the control of the accumulation responses.

oxidation is inhibited. Third, incorporation of free proline into protein is impaired and fourth, export of proline via the phloem is reduced (26). Upon relief of stress, proline levels decline rapidly in viable tissue due to oxidation via P-5-C and glutamate and due to incorporation into protein (e.g. 24,25).

A priori, it is not possible to classify any of these changes which occur during and after stress as adaptive, deleterious or incidental–although reasonable hypotheses based on the possible value of proline as an N-store, energy-store, NH_3-detoxification product etc. have been advanced (review:26). We therefore combined physiological and genetic approaches to determine whether proline accumulation in barley could be classed as an adaptive response. Our results indicated that it could not, but rather that it was an *incidental response* to the development of severe internal water dificit. This view has received recent support from Wyn Jones and Storey (32). Our evidence can be summarized as follows:

1) There were clear cultivar differences in the capacity to accumulate proline during water stress, as shown for 'Proctor' and 'Excelsior' barley in Fig. 4. Of the 2 cultivars, 'Proctor' (the more drought–susceptible) showed the higher proline accumulation rate which was associated with a more rapid decline in ψ_{leaf}. This faster drop in leaf water status in 'Proctor' probably resulted from the lower stomatal resistance and higher transpiration rate in wilted 'Proctor' leaves than in 'Excesior' (Fig. 4, inset table). 'Proctor' showed more severe leaf-killing during stress than did 'Excelsior', and much of the proline accumulated by both cultivars was located in the killed zone of the leaf–which would prevent its utilization upon rewatering (10).

2) The difference in proline accumulation rate between 'Proctor' and 'Excelsior' was heritable, because selection for high and low accumulation rates in the F_2 and F_3 progenies from a 'Proctor' × 'Excelsior' cross was effective. Within the F_3 population, which was segregating for the capacity to accumulate proline, proline accumula-

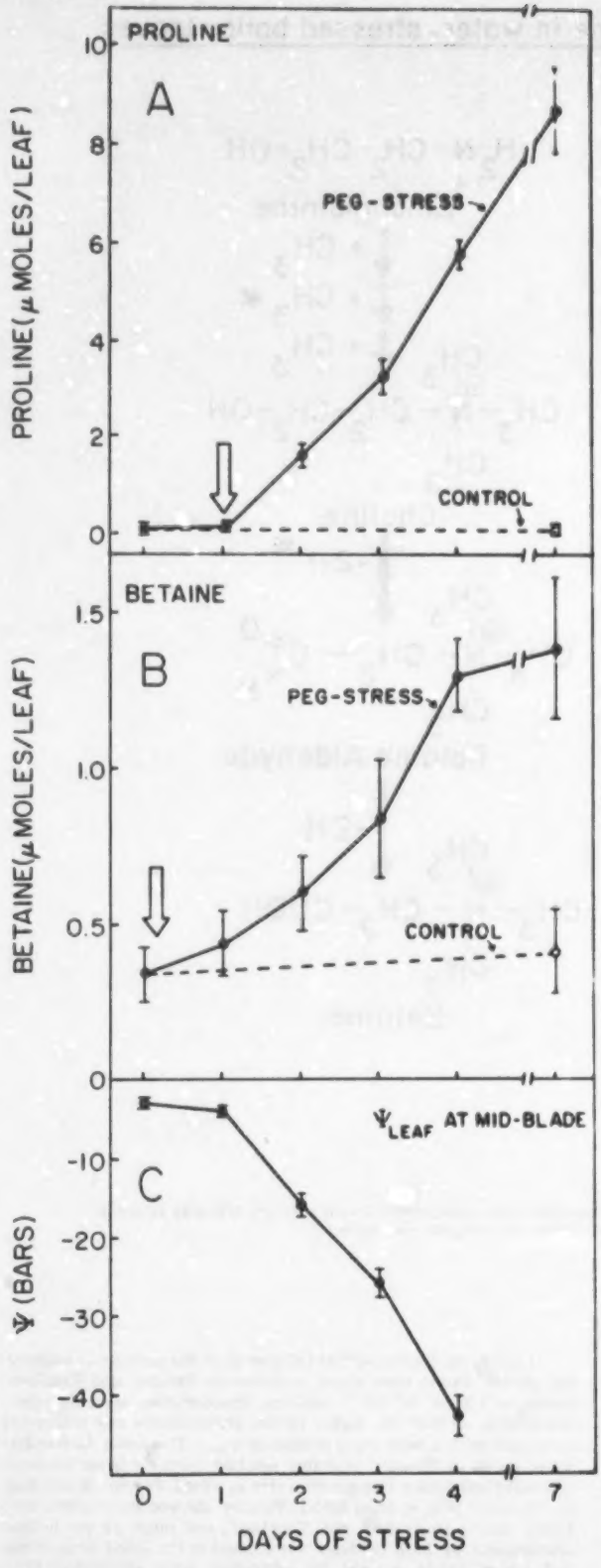
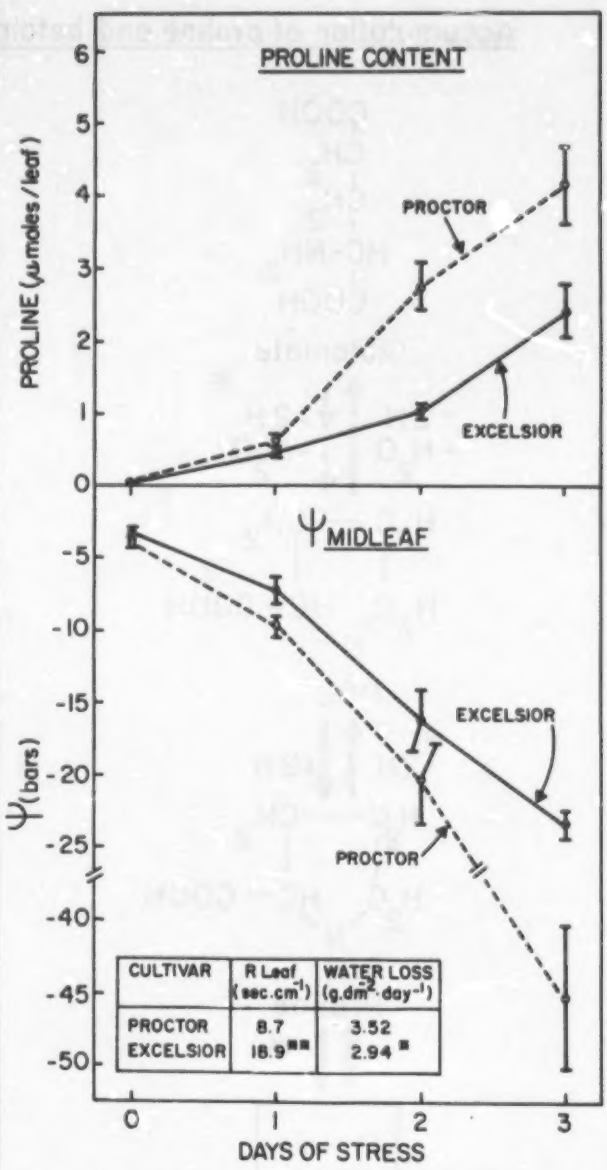


Fig. 3. Typical time courses for the decline of ψ_{leaf} and the accumulation of proline and betaine in second leaves of 'Proctor' barley seedlings, during a period of water stress. Stress was imposed by flooding the perlite rooting medium with polyethylene glycol 6000 (PEG) solution, $\psi_s = -19$ bars. Arrows in A and B indicate the earliest times at which net synthesis of [^{14}C]proline and [^{14}C]betaine from their respective precursors ([^{14}C]glutamate and [^{14}C]ethanolamine) was detectable in similar experiments.



CULTIVAR	R Leaf (sec.cm^{-1})	WATER LOSS (g.dm^{-2}.day^{-1})
PROCTOR	8.7	3.52
EXCELSIOR	18.9**	2.94 *

Fig. 4. Time courses of ψ_{leaf} and proline content in the second leaf blades of 2 barley cultivars during stress imposed as in Fig. 3. The inset table shows leaf diffusive resistance values (upper surface, second leaf blade) and transpiration rates (obtained by pot weighings) for the same pair of cultivars in a separate experiment. Asterisks (*,**) indicate differences significant at the 5% and 1% levels, respectively.

tion was *positively* associated with the extent of leaf-killing during stress. A *negative* association would be expected if the capacity to accumulate proline was of adaptive value (12).

3) The proline accumulated during stress was not significant as a soluble N– or energy–store (30,9), nor was it important in phloem-transport of N during stress, or as an end-product of NH_3-detoxification (13,29,30).

Betaine accumulation. The accumulation of betaine during stress has been much less studied than the accumulation of proline; the extent of genetic variability—particularly intraspecific variability—is uncertain, and it is not yet possible to classify betaine accumulation as an adapt-

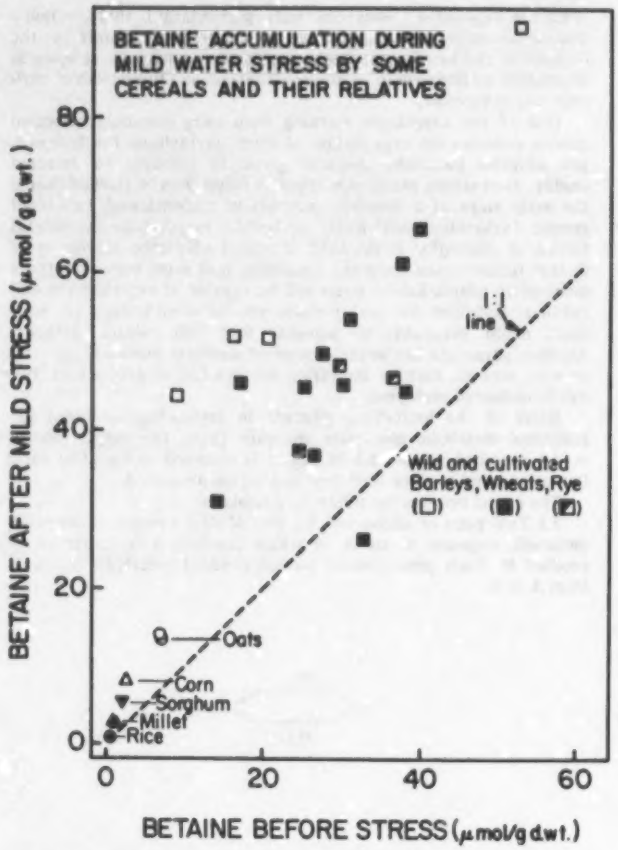


Fig. 5. Betaine contents of the shoots of some cereals and grasses before stress and after mild stress. Plants were grown in soil in the greenhouse to the 3- to 4-leaf stage, at which time stress was imposed by withholding irrigation for about 4 days. Betaine contents were determined by pyrolysis-gas chromatography (W.D. Hitz, unpublished results). Note that the 1:1 line represents a "no-accumulation" response, and that genotypes represented by points falling above the line accumulate betaine under stress.

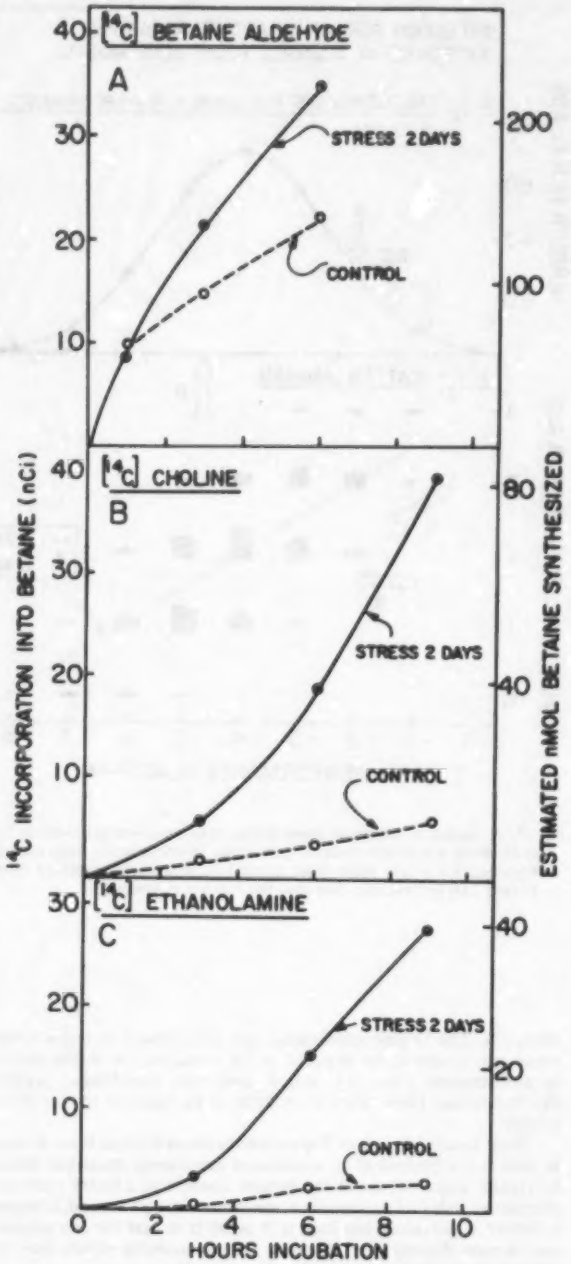


Fig. 6. Synthesis of [^{14}C]betaine from various [^{14}C] precursors by unstressed and stressed barley leaves. Labeled precursors (1 μmol/leaf) were supplied via the cut ends of attached second leaves. Control leaves were from well-watered plants and stressed leaves (ψ_{leaf} about −20 bars) were from PEG-treated plants. Specific radio-activities of precursors (nCi/nmol) were as follows: betaine aldehyde, 0.16; choline, 0.48; ethanolamine, 0.70.

ive, deleterious or incidental response to stress. However, among the cereals and grasses some interesting differences between proline and betaine accumulation can already be recognized.

Firstly, proline (a protein amino acid) is an essential metabolite whose pre-stress level is always low and tightly regulated, and proline accumulation is likely a universal response to stress among the grasses; on the other hand betaine seems to behave as a secondary metabolite which can be present at various levels or virtually absent before stress, and which does not necessarily accumulate during stress. Thus, the level of betaine in various grasses in the absence of stress spanned a wide range (Fig. 5, X-axis), which should be compared to the narrow (1 to 5 μ mol/g dry weight) range for proline. The accumulation of betaine upon moderate stress varied from little or none to a maximum of no more than about 5-fold; unlike proline, there were no instances where a low level before stress was associated with a high level after stress (Fig. 5).

Secondly, the causes for betaine accumulation are quite unlike those for proline accumulation (Fig. 2). Betaine is a metabolic end-product with little or no turnover, because there is no detectable betaine degradation before, during or after stress over a time-period of 2 to 7 days (11). Hence, accumulation involves only changes in rates of synthesis and export from the leaf. It appears that in stressed leaves the capacities for methylation of ethanolamine and for oxidation of choline increase markedly, while the last step in the biosynthetic pathway—oxidation of betaine aldehyde—is only slightly affected by stress (Fig. 6). In barley leaves subjected to sudden and severe water-stress, betaine accumulation may begin before proline accumulation. The arrows in Fig. 3 indicate that net [^{14}C]-betaine synthesis from [^{14}C]ethanolamine could be detected earlier than net [^{14}C]proline synthesis from [^{14}C]glutamate; see also (32).

Thirdly, because betaine is metabolically inert, betaine levels in viable tissue do not fall as rapidly as proline levels upon relief of

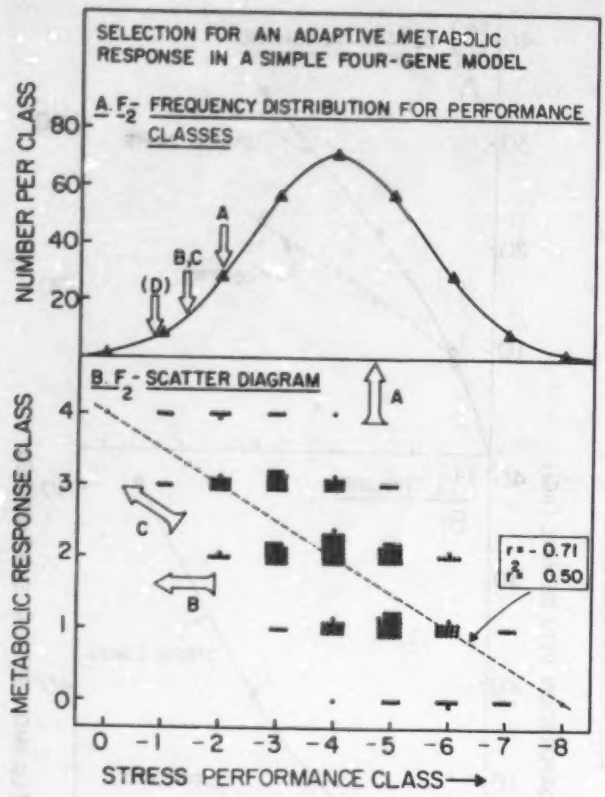


Fig. 7. A model to illustrate some limitations involved in selecting for individual metabolic traits. Each point in the scatter diagram (B) represents a single plant from a total segregating population numbering 256 individuals. See text for furtner explanation.

stress (8). The betaine synthesized and accumulated in leaves during stress can, however, be disposed of by translocation in the phloem to meristematic sinks (11, and J. Ladyman, unpublished results); like the leaves, these sinks show little or no capacity to metabolize betaine.

These results encourage 2 speculations about betaine. First, because betaine is synthesized at an accelerated rate during stress but cannot be rapidly degraded, could the betaine content of a barley plant be a cumulative index of its internal water-stress history? Second, if betaine is indeed a secondary product, is it possible to test for any adaptive significance directly by obtaining barley mutants which lack the betaine-accumulation response?

Proline and betaine accumulation as practical tools? In principle, proline has some potential as a metabolic symptom of stress, but the expense of testing for proline may not be worthwhile inasmuch as stressed tissues with high proline levels will probably show obvious visual stress symptoms (e.g. leaf rolling or firing) which can be scored more easily. Betaine accumulation holds promise as an indicator of stress history, and is currently under evaluation in our laboratory for this and for possible adaptive value.

Metabolic responses and plant breeding

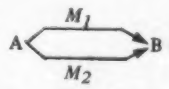
If adaptive metabolic responses to stress can be identified and if useful genetic variation for these characters can be detected by routine tests, it becomes feasible to practice selection for such characters in breeding programs. (Similarly, deleterious or incidental responses to stress could be employed as stress symptoms, with selection *against* symptom expression.) Metabolic tests—particularly if they are non-destructive—might thus supplement the techniques currently at the disposal of the breeder who seeks to achieve genetic advance towards adaptation to stress (9,26). Certain advantages and limitations of such tests can be foreseen.

One of the advantages accruing from using metabolic selection criteria concerns the exploitation of exotic germplasm. For instance, and adaptive metabolic character could in principle be detected readily, even among exotic genotypes. It could then be tracked during the early steps of a breeding program in material with an overall genetic background sufficiently outlandish to preclude meaningful testing of adaptation in the field. A related advantage—for the more distant future—arises from the possibility that some metabolic traits involved in adaptation to stress will be capable of expression in cell cultures. Selection for, and perhaps genetic modification of, such traits might eventually be achieved with cell culture methods. Another advantage lies in the chance of screening materials between growing seasons, thereby increasing the number of generations that can be evaluated each year.

Some of the limitations inherent in attempting to breed for individual metabolic characters are quite plain. The simple genetic model described below and in Fig. 7 is intended to highlight such limitations, and to show how they may be circumvented.

The model involves the following postulates:

1.) Two pairs of alleles (M_1M_1' and M_2M_2') control an adaptive metabolic response to stress, in which reactant A is converted to product B. Each gene governs an independent metabolic pathway from A to B:



Each dose of M_1 or M_2, contributes +1 unit to the capacity to convert A to B during stress; M_1' and M_2' each contribute 0 units. There are thus 5 possible classes of phenotype for the adaptive synthesis of B in response to stress, from 0 ($M_1'M_1'M_2'M_2'$) to 4 ($M_1M_1M_2M_2$).

2.) Overall plant performance under stress (i.e. survival, growth or yield) depends upon the behavior of every character in the genome. In addition to M_1 and M_2, the model assumes that *just 2* other pairs of alleles (S_1S_1' and S_2S_2') act independently of each other and of the metabolic pathways controlled by M_1 and M_2 in determining performance under stress. (S_1 and S_2 could be thought of as genes governing adaptive morphological or phenological characters). Each dose of S_1' or S_2' contributes −1 unit to overall stress performance, as does each dose of M_1' or M_2'. S_1, S_2, M_1 and M_2 each contribute zero units. There are thus 9 possible phenotype classes for stress performance, ranging from 0 (best performance, minimum symptoms of stress: $M_1M_1\ M_2M_2\ S_1S_1S_2S_2$) to −8 (poorest performance, maximum symptoms of stress: $M_1'M_1'M_2'M_2'\ S_1'S_1'S_2'S_2'$).

3.) Linkage and epistasis are absent; heritabilities are 100%.

4.) A cross is made between the parental genotypes $M_1M_1M_2M_2$ to be incorporated into current cultivar P_2.

5.) The F_2 generation from this cross is screened for the metabolic response in a non-destructive seedling-stage test by which the 5 metabolic (M) phenotype classes are distinguished (Fig. 7B, Y-axis).

6.) The F_2 plants tested are subsequently evaluated for overall performance under stress in the field, in which the 9 performance (M +S) phenotype classes are distinguished (Fig. 7B, X-axis).

Fig. 7B is a scatter diagram showing the 256 possible genotypes present in the F_2. The following 3 limitations are apparent from this diagram. First, it is unrealistic to expect an individual metabolic trait to be highly correlated with overall crop performance under stress. Even though in the model 50% of the performance under stress is accounted for by the adaptive trait and there are no environmental interactions, considerable scatter is evident in the F_2 population and the anticipated correlation between adaptive trait and overall performance is *not strong* (i.e. $r = -0.71$, $r^2 = 0.50$). The weakness of such relationships is mathematically inevitable and obviously increases as the number of genes controlling stress performance multiplies in relation to the number controlling the metabolic trait. Because, in practice, very many genes affect stress performance, it is unlikely that selecting *only* for a single adaptive metabolic trait could be very

effective in improving overall stress performance. To illustrate this point, 3 alternate breeding strategies are compared in the model. The arrows in Fig. 7A indicate the genetic advance in stress performance expected from a single cycle of selection with a selection intensity of 6%, using 3 selection strategies:

a) for the metabolic trait alone (strategy A);

b) for stress performance (strategy B);

c) for a combination of both metabolic trait and overall performance (strategy C).

Note that the *least* effective strategy is A, and ...at C can be shown to be superior to B, because C secures a higher concentration of the desired genes M_1 and M_2 in the selected population.

The other 2 limitations of breeding for individual metabolic traits concern numbers. To achieve modest advances in stress performance by strategies A, B and C, many individuals (>256) have to be tested, and 94% of these must be discarded. This presents the double disadvantage of high testing costs, and serious incompatibility with other breeding objectives. Since breeding programs simultaneously have to keep many traits besides adaptation to stress in focus, more than 6% of a segregating population must often be available for selection related to these other breeding objectives.

Fortunately, the drawbacks of low correlations, high costs and prohibitive selective intensities may be largely overcome by designing breeding strategies *around* metabolic testing procedures. Thus, in the model above, an F_2 population is not the most appropriate place to apply a metabolic selection criterion. An alternative could be to select for both adaptive trait and stress performance with an intensity of about 20%, first on the progeny of the F_1 backcross to P_2 (the agronomically desirable parent), and then in a second cycle on the selfed progeny of the selected individuals. It can be shown that this strategy (D in Fig. 7A) should achieve more genetic advance in stress performance per unit number of individuals tested than the strategies A, B or C in which an F_2 population is screened. Further, the backcrossing step and the less stringent, 2-stage selection improve the chances of recovering desired agronomic traits in the tested populations.

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STRESS ETHYLENE PRODUCTION—A MEASURE OF PLANT RESPONSE TO STRESS

David T. Tingey
U.S. Environmental Protection Agency, Corvallis, OR 97330

Data on plant response to stress are used to implement the Toxic Substances Control Act, the Clean Air Act, and other federal regulations. Phytotoxicological data are essential in assessing the environmental hazards inherent in waste, sludge and/or dredge spoil disposal methods and in evaluating the potential or existing impacts of various industrial and/or energy processes.

Any method used to quantify plant stress should exhibit certain characteristics. It should yield objective data. The response should be sensitive with a relatively broad response range. It should be relatively nonspecific to minimize false negatives. The method should produce results that are reproducible among researchers, locations and through time. A simple technique is preferred to reduce error and minimize effort. Any plant response can be a measure of plant stress, but the response parameter should have biological significance, or be related to a biologically significant response.

Stress ethylene

Plants normally produce small amounts of hormonal ethylene, but when subjected to stress, they increase production of ethylene. This surge in ethylene production is frequently referred to as wound or stress-induced ethylene. Ethylene emissions can increase from 2 to more than 50 times the basal level, depending on the stress imposed and the sensitivity of the plant tissue to that stress. Injured, not dead tissue is the site of stress ethylene production and it is apparently produced by the same metabolic pathway that produces hormonal ethylene (1,6). Also, some stress ethylene may result from the peroxidation of linolenate (6). The induction, biosynthesis, and the possible significance of stress ehtylene has been reviewed recently (6).

Stress ethylene is induced, in plants infected with pathogenic microorganisms and it has been implicated in pathogenesis (8,14). Ethylene production is stimulated by physical factors such as wounding, bruising, chilling, freezing, and radiation (1,6,7). Gaseous air pollutants also elicit stress ethylene production (1,2,4,9,11,12).

Because stress ethylene is induced by a wide array of biotic and abiotic stresses, it was selected as a response parameter to quantify plant stress induced by chemicals applied to the plant in either the aqueous or gaseous phase. This paper summarizes the results of a series of experiments which illustrate the use of stress ethylene production to assess plant response to stress.

Methodology

Test solutions were applied to plant roots by dispensing a measured volume of various concentrations onto the surface of the plant growth medium or by spraying a measured amount onto the foliage. Exposure chambers (11,12) were used to fumigate plants with gases. Following the application of the stress, the plants were encapsulated in either Saran or B-620 plastic bags which have a low permeability to ethylene. The bags were supported with wire frames to hold them away from the foliage and provided a constant volume. Encapsulated plants were usually incubated at 26°C in dark for at least 1 hr; in some studies the incubation lasted 22 hr. The ethylene emitted from the plant accumulated in the bag head space and was sampled by inserting a 1-ml gas-tight syringe through the bag. The ethylene was separated and quantified with a gas chromatograph using a flame ionization detector (11,12).

Preliminary studies indicated that the variance of ethylene measurements was positively correlated with the mean ehtylene concentration. Therefore, the data were transformed to the respective natural logarithms to stabilize the variability (10). All calculations were performed on the transformed data.

Stress ethylene–dose response relationship

Over a large range of concentrations, stress ethylene production follows the Arndt-Schulz Law (5) which states that there is a universal tendency for low concentrations of toxicants to stimulate biological processes and for higher concentrations to depress them. Also, in sensitive species, biological processes are stimulated at lower concentrations than in tolerant species (5). To illustrate the response of stress ethylene production to a range of chemical stresses, test compounds at various concentrations in the aqueous ($CdCl_2$, Endothal, Phytar 560) and gaseous (O_3Cl_2) phases were applied to plant roots and foliage. The toxicant concentrations were chosen so that ethylene production would be in the linear portion of the response curve.

A concentration series of $CdCl_2$ solutions applied to the roots of bean plants elicited a significant increase in stress ethylene production (Fig. 1). The increase was approximately linear over the range of 0.1 to at least 10 mM for both the 5- and 10-hr sampling periods following cadmium application. Ethylene production in the plants treated with 0.1 mM $CdCl_2$ was not significantly different from the control plants.

Application of a concentration series of toxicant solutions to bean plant foliage induced significant increases in stress ethylene production, increases which were approximately linear with concentration (Fig. 2). Both compounds tested (the herbicides Endothal and Phytar 560) induced stress ethylene at concentrations well below their recommended field application rates and at concentrations that did not induce visible foliar injury. However, Endothal elicited stress ethylene production at a lower concentration and caused a larger increase than did Phytar 560.

When various plant species with diverse genetic backgrounds were exposed to ozone for 2 hr, ethylene production increased proportionally to ozone concentration (12). At concentrations of 0.75 µl/liter stress ethylene production was 13, 4 and 1.5 times above the basal levels for pine, tomato and corn, respectively (12). Similarly, there was a high correlation between stress ethylene production and the sulfur dioxide concentrations to which cucurbits leaves were exposed (4).

These data support the concept that stress ethylene production is proportional (up to a limit) to the concentration of toxicant to which the tissue is exposed. The increase occurred regardless of whether the stress was applied in the aqueous phase to the roots or

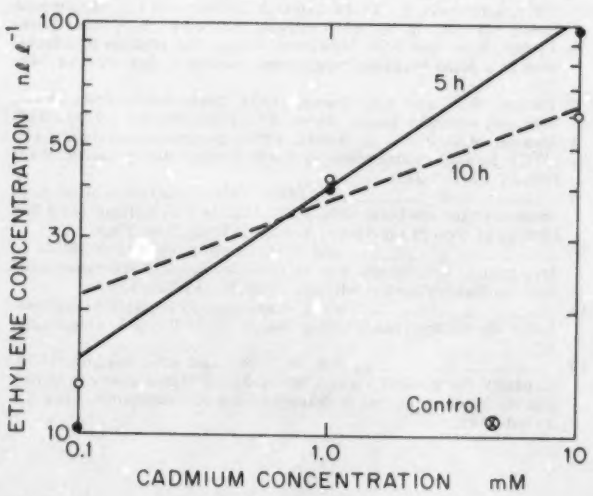

Fig. 1. Cadmium chloride induction of stress ethylene in bean plants. Test solutions (50 ml) were applied to the surface of plant growth media. Plants were encapsulated 5 and 10 hr after application and incubated for 2 hr. Each mean is based on 16 observations.

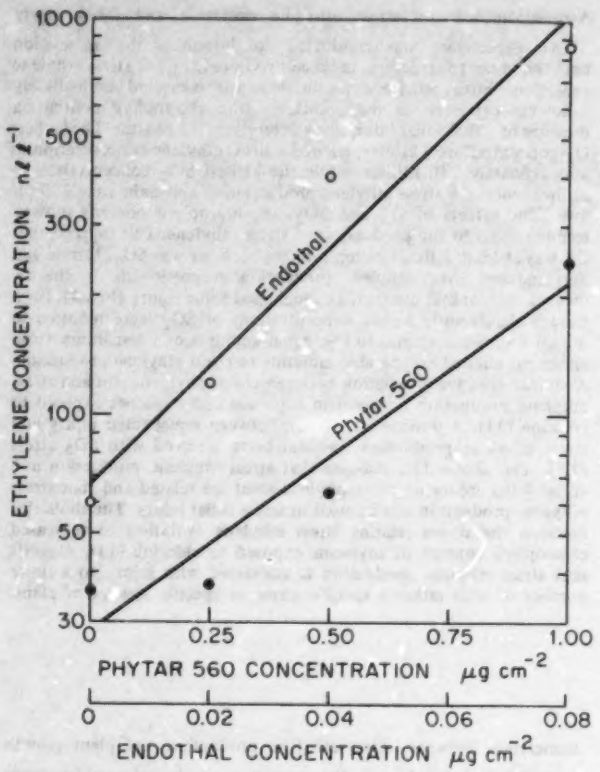

Fig. 2. Herbicides increase stress ethylene production in bean plants. Test solutions were sprayed onto the foliage to yield the concentration of foliar depositions shown. Each mean is based on 9 observations (Data from C. R. Thompson, personal communication).

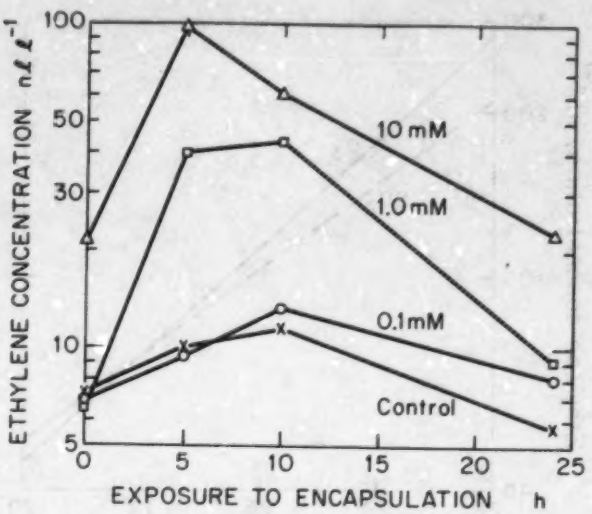

Fig. 3. Duration of cadmium induced stress ethylene producion in beans. Each plant was encapsulated for 2 hr at either 0, 5, 10 or 24 hr after cadmium application to the roots. Each mean is based on 16 observations.

foliage, or in the gaseous phase to the foliage. This increase in stress-induced ethylene production can be modeled using the following equation:

Log_e [ethylene concentration] = Log_e A + B [concentration of stress]. In this equation, A is an estimate of the ethylene production from non-stressed (control) plants and B is the slope parameter which is a measure of the increase in stress-induced ethylene production in relation to the stress concentration. The slope parameter can measure the sensitivity of the plant tissue to a specific stress as determined by the rate of ethylene production. A large slope parameter indicates that the plants are sensitive, while a small slope parameter indicates less sensitivity to the stress.

The slope parameter B was used to rank the relative sensitivity of 29 species and/or cultivars to ozone (12). O_3-enhanced stress ethylene production in all plants, but at differing rates; slope parameters ranged from a high of 3.46 to a low of 0.12 for ponderosa pine and marigold, respectively. Ponderosa pine, eucalyptus and soybean were ranked as most sensitive, while holly, squash and marigold were the least sensitive. In addition to identifying differences in sensitivity among species, the slope parameter ranking technique was also able to detect differential sensitivity within tomato, begonia and lupine cultivars. These data indicate the usefulness of ranking plants according to their relative sensitivity, based on stress ethylene production.

Similarly, the application of a series of aqueous sprays at different toxicant concentrations to the foliage of kidney beans caused differential increases in stress ethylene production, depending on the test compound. The slope parameters computed from the linear regression model were 33.46, 2.19 and 0.02 for Endothal, Phytar 560 and NaF, respectively (C. R. Thompson, personal communication). These slope parameters were used to differentiate the relative phytotoxicity of the test compounds and to provide a relative sensitivity ranking of beans to the compounds.

Reproducibility

A reliable measure of plant response to stress should be reproducible through time with minimal variation. The reproducibility of the slope parameter was tested by conducting a series of independent ozone exposures on plants of the same species during several months and by estimating the slope parameter (12). Based on repeated trials, the slope parameter estimates for soybean, pine and potato were within their respective 95% confidence interval estimates, suggesting that the estimates were reproducible.

To assess the reproducibility of the plant response to $CdCl_2$ stress, beans were treated with either deionized water or a solution of 10 mM $CdCl_2$ applied to the plant growth medium. The plants were grown in a greenhouse from October through May and stressed 14 days after planting. During this period, the mean slope parameter estimate was 0.25 with a 14% coefficient of variation. The only significant deviation occurred during January when the slope parameter was 0.14, which corresponded to the period of minimum plant growth. This suggests that if plant growth conditions were reproducible, the slope parameter for chemical stress to root systems would also be reproducible.

Duration of stress ethylene production

Studies were conducted to determine the duration of stress ethylene evolution from a single stress application. Several concentrations of $CdCl_2$ solutions were applied to the plant growth medium and ethylene production was determined 0, 5, 10 and 24 hr after stress application (Fig. 3). The ethylene production rate increased rapidly following application, peaking within 5 to 10 hr, depending on the $CdCl_2$ concentration. Following the maxima, stress ethylene production decreased until the production at 24 hr was not significantly different than at 0 hr. Subsequent studies showed that repeated daily application of $CdCl_2$ stimulated stress ethylene formation each time. The response to a series of individual $CdCl_2$ stresses was maintained over the two-week study period, indicating that the stress ethylene biosynthetic pathway was still functional.

To determine the duration of stress ethylene production induced by gases, plants were exposed to chlorine for 2 hr and then encapsulated for 4 hr 0, 1, 2, 3 or 4 days after exposure (11). Chlorine (0.5 μl/liter) significantly increases stress ethylene production on the day of exposure. Within 24 hr, the rate of stress-induced ethylene production had returned to the control level and remained there for the duration of the study. Similar results were obtained from O_3 exposures (12) in which the maximum ethylene production occurred

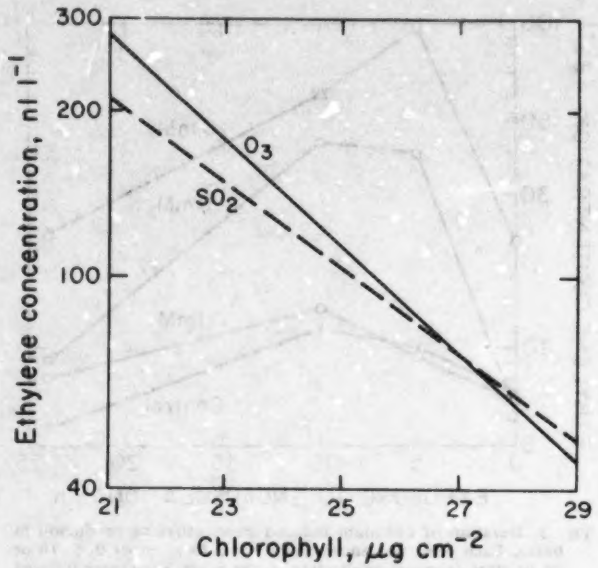

Fig. 4. The relationship between stress ethylene production and foliar injury. Data from 60 plants were used to develop each linear regression line.

Table 1. Site of stress ethylene production (nl/liter).[z]

Cadmium concn (μM)	Ethylene (nl/liter)	
	Leaves	Roots
0	19	32
10	189	345

[z]Plants were encapsulated 5 hr after cadmium application and incubated for 2 hr in the dark prior to measuring ethylene. Even though the plant parts were encapsulated separately, the plants remained intact.

within the first 1 to 4 hr following exposure, with a return to the control on subsequent days. Daily O_3 exposures over a two-week period stimulated the stress ethylene production daily. This ability to respond to a series of individual ozone stresses indicated that the plant still maintained the metabolic capacity to produce stress ethylene. Uno and Miyake (13) reported that stress ethylene production peaked within 4 hr after exposure. Experiments with SO_2 stress reported peak ethylene production within 1 hr (2) or within 4 to 8 hr (9) after exposure, followed by a decline to the basal level.

The decrease in stress ethylene production observed during 24 hr with $CdCl_2$, O_3 and Cl_2 stresses suggests that the plants either repair the system or compensate for the stress-induced perturbations and/or sequester the stress inducing agent. The plant's ability to respond to daily stress applications ($CdCl_2$ or O_3) over a 2-week period, by releasing enhanced daily levels of ethylene, suggests that the plants maintain the mechanism of stress ethylene production.

Site of stress ethylene production

To determine the site of stress ethylene production, a 10 mM solution of $CdCl_2$ was applied to the growth medium of bean plants. The pot-root mass and the plant foliage were encapsulated separately and incubated (Table 1). Significant ethylene increases were measured around the roots and the foliage, indicating that both sites produce stress ethylene. This was expected because cadmium can accumulate in both tissues. Similar studies with ozone detected enhanced ethylene production only around the foliage. This suggests that O_3 stress is not translocated throughout the plant, because O_3 cannot diffuse any significant distance into the plant rooting medium (3).

Association between stress ethylene evolution and foliar injury

An experiment was conducted to determine the association between visual foliar injury, induced by O_3 or SO_2, and stress ethylene evolution. Stress ethylene production was measured immediately following exposure to the pollutants; the chlorophyll content, a measure of visual foliar injury, was determined 72 hr later. The highest O_3 concentration, 1 μl/liter, elicited a stress ethylene concentration of approximately 240 nl/liter, while the highest SO_2 concentration, 3 μl/liter, elicited a stress ethylene production of approximately 190 nl/liter. The effects of O_3 and SO_2 on chlorophyll content showed trends similar to the production of stress ethylene. This suggests that O_3 was at least 3 times as toxic to soybeans as was SO_2. Ozone and SO_2 induced stress ethylene production proportionally to the decreased chlorophyll content, i.e., increased foliar injury (Fig. 4). Even though significantly higher concentrations of SO_2 were required to obtain a similar response to O_3, equal amounts of foliar injury from either gas elicited comparable amounts of stress ethylene production. A similar negative association between chlorophyll content and stress ethylene production occurred in soybeans and tomatoes exposed to chlorine (11). A positive association between visual foliar injury and stress ethylene production has also been observed with SO_2 stress (2,4). The above data indicate that stress ethylene production and visual foliar injury or chlorophyll content are related and that stress ethylene production can be used to assess foliar injury. The similarity between the slopes relating stress ethylene evolution to decreased chlorophyll content of soybeans exposed to chlorine (11), suggests that stress ethylene produciton is associated with injury to a finite number of cells rather a specific stress or specific species of plant.

Association between stress ethylene production and plant growth

Ethylene is involved in the regulation of physiological processes such as leaf expansion, leaf abscission, senescence, and the activity of several enzymes. Thus, it can significantly affect plant growth and yield (1). Two separate experiments were conducted using either $CdCl_2$ or O_3 to test the hypothesis that elevated stress ethylene production was associated with decreased plant growth.

Bean plants were treated with $CdCl_2$ solutions (10 to 500 μM) applied to the surface of the plant growth medium daily for 2 weeks. Stress ethylene production was measured for each plant prior to the daily harvests and then total plant dry weights were determined. Stress ethylene production increased proportionally to the cadmium concentration over the course of the study. At each harvest the percent deviation of the test plants from the average of the control plants was determined for plant growth and stress ethylene production to illustrate the association between stress ethylene production and growth (Fig. 5). Plants that were larger than the mean of the controls produced less ethylene than the controls. Conversely, plants smaller than the controls tended to produce more stress ethylene, indicating a significant negative association between stress ethylene production and plant growth.

A conceptually similar experiment was conducted to determine if there were an association between ozone-induced stress ethylene production and plant growth. Zinnia plants were exposed to either charcoal-filtered air of 0.15 or 0.5 μl/liter O_3 treatments 5 hr a day for 10 days. Stress ethylene production was measured for each plant prior to harvesting and then total plant dry weight was determined. The stress ethylene production increased above the control level within about 4 and 2 days for the low and high O_3 treatments, respectively. Over the course of the study, the mean ethylene concentrations increased in proportion to the O_3 concentration from 650 pl cm^{-2} of leaf area/hr for the controls to 725 and 1325 for the 0.15 and 0.50 μl/liter treatments, respectively. The same procedure used in the cadmium study cited above, was used to illustrate the association between plant growth and stress ethylene production (Fig. 6). There was a highly significant negative association between stress ethylene production and plant growth. Plants that were larger than their respective controls produced less stress ethylene, while plants that were smaller than their respective controls produced significantly more ethylene than their controls.

A highly significant negative association between stress ethylene production induced by either $CdCl_2$ or O_3 and plant growth was found. This suggests that the stress-induced reductions in plant growth were associated with elevated ethylene levels and may, in part, result from the increased production of stress ethylene.

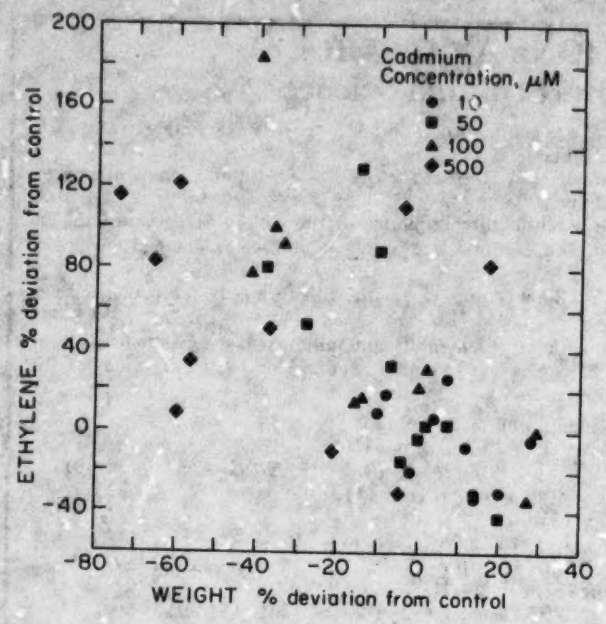

Fig. 5. Association between cadmium induced stress ethylene and the growth of bean plants. Each mean is based on 4 observations.

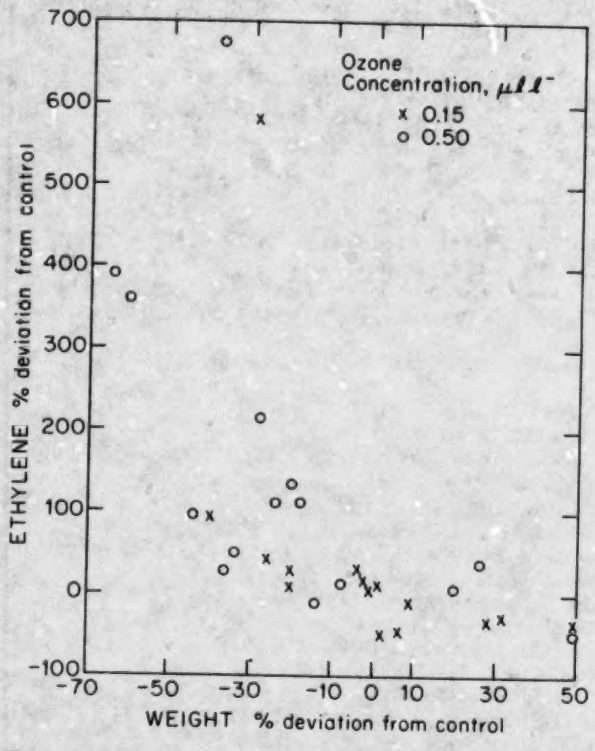

Fig. 6. Association between ozone induced stress ethylene and the growth of zinnia plants. Each mean is based on 4 observations.

Summary

The results of the experiments indicate that stress ethylene production is dependent on the stress concentration (up to a limit) from a wide variety of test chemicals applied either in the aqueous or gaseous phases. The production of stress ethylene is a short-lived phenomenon, peaking quickly after stress application and returning to control levels with 24 hr. Plants are capable of responding to repeated stress applications, which indicates that the tissue maintains its capacity to produce stress ethylene. Stress ethylene production is associated with visual injury and reduced plant growth, suggesting that stress ethylene may provide a reliable bioassay for determining the effects of a variety of stresses on plants.

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